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Nucleosome Positioning in *Saccharomyces cerevisiae*

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INTRODUCTION

In eukaryotic cells, long linear stretches of DNA must be contained within the very small space of the nucleus. Every human cell, for example, contains about 2 m of linear DNA, whereas the diameter of the nucleus is only 5 to 20 μm (135). This creates a packaging problem that all eukaryotic cells are faced with: long molecules of stiff, negatively charged DNA need to be folded into the confined space of the nucleus. Moreover, the folded DNA has to remain accessible for various cellular processes, such as replication and transcription. To achieve this, cells coil DNA around histones, barrel-like octamers of highly basic proteins that favor binding to the negatively charged DNA polymer. By neutralizing the negative charges and wrapping the DNA, the histone complex allows the DNA to be condensed about 10,000-fold. The protein-DNA complex that arises by the spooling of DNA around histones is known as chromatin (5, 82) (Fig. 1).

The basic repeating unit of chromatin is the nucleosome, consisting of 147 bp of DNA wrapped approximately 1.65 times around the histone protein octamer (48, 82, 106, 122, 125). The histone octamer contains two copies of each of the four canonical or core histones (H2A, H2B, H3, and H4) (82). Nucleosomes are joined by short stretches of DNA that run between them, referred to as linker DNA, which is bound by

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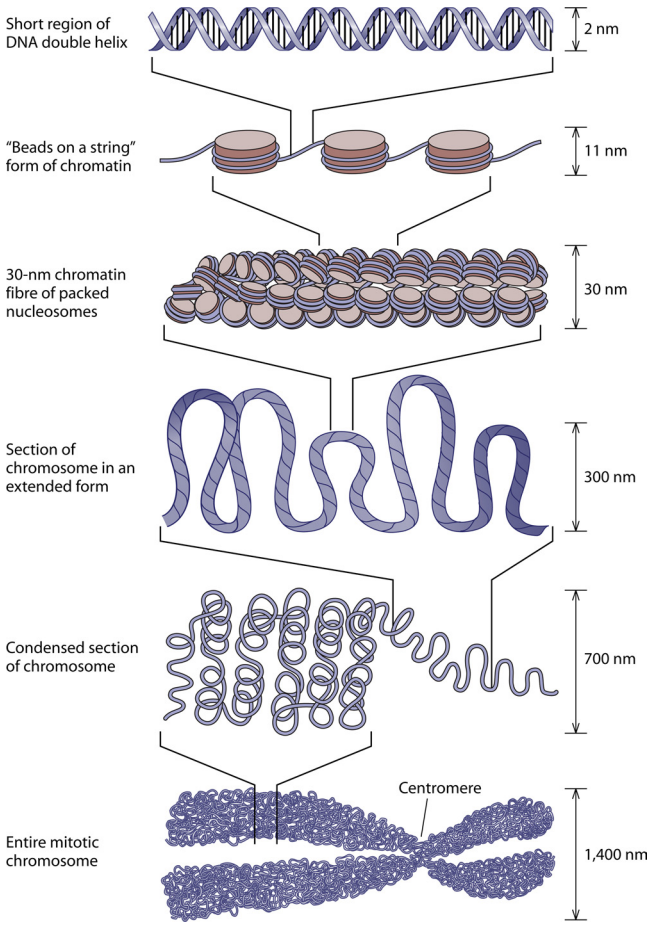

FIG. 1. Chromatin structure. DNA is wrapped around a histone octamer to form nucleosomes. Nucleosomes are connected by stretches of linker DNA. This basic nucleosome structure is folded into a fiber-like structure of about 30 nm in diameter. These 30-nm fibers are further compacted into higher-order structures, which have not been characterized in detail. (Adapted from reference 45a with permission of Macmillan Publishers Ltd., copyright 2003.)

linker histone H1 (125). Each histone protein consists of a globular domain and flexible, relatively unstructured "histone tails" that protrude from the nucleosome surface (106).

Nucleosomes are arranged as a linear array along the DNA polymer, which makes them appear as "beads on a string" by electron microscopy, with the string being formed by stretches of linker DNA in between consecutive nucleosomes (123). This beads-on-a-string pattern represents a primary packing level, and further condensation is achieved through the formation of the more compact and repressive 30-nm chromatin fiber (secondary level) and several more levels of higher-order chromatin organization (reviewed in reference 105) (Fig. 1).

Biological Role of Nucleosomes—More than Just DNA Packaging

Besides being a packaging unit, nucleosomes play crucial regulatory roles in many cellular processes, including gene expression, chromosome segregation, and DNA replication, repair, and recombination by controlling DNA accessibility. Indeed, the chromatin structure makes certain loci less accessible because the DNA is wrapped tightly around a nucleosome, whereas linker regions are much more accessible. Moreover, the chromatin structure is highly dynamic, and the remodeling of nucleosomes results in an altered DNA accessibility. Differences between individual nucleosomes also offer opportunities for regulation. Histone variants can take the place of core histones; for example, histone variant H2A.Z can replace core histone H2A, thus forming distinct octamers that may serve specific regulatory roles (reviewed in references 77, 147, and 167). The histone tails also contribute to nucleosome variability, as many of the amino acid residues are subject to extensive posttranslational modifications (e.g., acetylation and methylation), which change the physical properties of the nucleosome and sometimes elicit *trans* effects by recruiting other regulatory modification-binding proteins to the chromatin (reviewed in references 84 and 113). Together, these mechanisms create variation in the chromatin polymer that is crucial for the regulation of all of the processes mentioned above.

Given the influence that nucleosomes have on several vital biological processes, the correct and exact positioning of nucleosomes is critical for proper genome functioning. In this review, we discuss our current understanding of signals that direct nucleosomal organization. Histone octamers do not bind the DNA randomly, and many major factors that affect nucleosome positioning have been identified; however, these factors are rather complex and make it difficult to understand and predict the exact position of each nucleosome in a genome. We first focus on so-called *cis* factors, i.e., characteristics of the local DNA that influence nucleosome positions. Special emphasis is put on genome-wide studies, as they have transformed the chromatin field in recent years. Next, we discuss *trans* factors contributing to chromatin organization, including a variety of proteins and protein complexes that modify nucleosome positions by acting on or interacting with chromatin. At the border between *cis* and *trans* factors are the effects of neighboring nucleosomes and higher-order chromatin structures. A separate section will discuss how these *cis* and *trans* factors contribute to elegantly coordinated regulatory effects, illustrated by several examples from the literature of how the nucleosome organization affects the gene expression activity. Throughout this review, we focus primarily on the chromatin structure in *Saccharomyces cerevisiae*, which has been studied most extensively and serves as a model for higher eukaryotes; however, in a final section, we explore differences between the model yeast and other organisms.

GENOME-SCALE NUCLEOSOME MAPPING

It has been clear for a long time that chromatin organization is directed partly by positioning signals encoded in the local DNA sequence (i.e., "in *cis*") (101, 149, 179, 180). Early studies showed that variations between DNA sequences play a role in nucleosome positioning because certain sequences intrinsically favor or disfavor nucleosome formation (37, 101, 149, 179, 180, 212). Due to the sharp bending that the DNA helix is forced to undergo in order to wrap around the histone octamer, it was reasonable to conclude from those studies that the physical properties of the DNA sequence would affect nucleosome positioning. Recently, we have been able to test these ideas di-

rectly and rigorously using new technologies designed to measure nucleosome positions on a genome-wide scale. Using these data, follow-up analyses have allowed computational algorithms and models to deduce which sequences show a higher or lower tendency to form nucleosomes. To begin the discussion of these analyses, we first give a brief overview of different genome-wide nucleosome-mapping technologies that have facilitated these breakthroughs.

Basic Strategies for Genome-Wide Nucleosome Mapping

Most nucleosome-mapping techniques rely on the same basic principles (Fig. 2). Essentially, chromatin is first digested by a nuclease into mononucleosomal DNA fragments. Linker DNA in between nucleosomes is more accessible to endonucleases, which therefore preferentially hydrolyze linker DNA, while nucleosome-covered sequences are protected from digestion. Next, the undigested, nucleosome-covered DNA fragments are isolated, purified, and analyzed.

Nucleosomes have been mapped both *in vivo* and *in vitro*. While *in vivo* studies give a detailed picture of the chromatin organization in living cells, *in vitro* studies have the advantage that they focus solely on DNA-encoded sequence preferences by excluding the effects of other *trans*-mediated determinants. *In vitro*, chromatin is reconstituted by using purified histone octamers that are assembled onto purified genomic DNA either by salt dialysis (168) or by using purified chromatin assembly proteins. An example of the latter is the *Drosophila melanogaster* ACF protein, which mediates nucleosome assembly *in vivo* and can be used to achieve chromatin assembly *in vitro* in an ATP-dependent manner (49). *In vivo* studies are generally carried out by cross-linking histones to the nucleosomal DNA in living cells by using formaldehyde, thereby trapping nucleosomes at their *in vivo* locations (138).

Chromatin fractionation is then achieved by using a nuclease to digest the nonprotected linker DNA. Micrococcal nuclease (MNase) is most typically used, as it has a distinct preference for digesting linker DNA, while nucleosomal DNA is (at least partly) protected from MNase digestion. In *in vivo* experiments, MNase digestion is carried out with permeabilized cells (i.e., spheroplasts) that originate after the partial enzymatic digestion of the cell wall using zymolyase. Digested chromatin is subsequently isolated from the cells by using gentle cell lysis followed by DNA extraction (138). Chromatin digestion is sometimes followed by immunoprecipitation with antibodies against tagged histones or specific histone modifications, thereby enriching for a particular subset of nucleosomes for subsequent analysis. The resulting fragments of nucleosomal DNA are deproteinized and purified, and ~150-bp-long fragments are size selected by agarose gel electrophoresis (138) (Fig. 2).

In a final step, the collected DNA fragments are identified, and the enrichment of each position is analyzed. Many techniques provide different levels of resolution and accuracy for nucleosome positioning analysis. However, all methods generally proceed by measuring which sequences in the MNase DNA preparation are enriched relative to others. Normalization to the same genomic DNA from which all histones were removed by protease digestion before analysis is essential to account for biases with each method.

Several techniques were developed to investigate single genomic loci, including a Southern blotting assay ("indirect end labeling"), primer and monomer extension (reviewed in reference 26), and tiling (quantitative) PCR (90, 157). More recently, genome-scale studies have used microarray hybridization or high-throughput sequencing to explore nucleosome positions. In microarray experiments, fluorescently labeled nucleosomal DNA fragments and a genomic DNA control are hybridized onto a microarray. For each probe on the array, the hybridization intensity for the nucleosomal DNA is plotted against the value measured for the control sample. Nucleosome-occupied regions have a higher-than-average ratio value, whereas nucleosome-depleted regions (NDRs) have a lower-than-average ratio value.

While the resolution of microarray experiments is limited by both probe size and probe spacing, high-throughput sequencing experiments allow the mapping of individual nucleosomes with single-base-pair resolution. Here, mononucleosomal DNA fragments are sequenced in a massive parallel sequencing experiment. Data analysis starts by the mapping of the sequence reads onto a reference genome, taking the number of sequence reads as a measure of the enrichment of undigested DNA. Here too, normalization to the same genomic DNA is crucial to account for biases in the sequencing technology. Indeed, it has been established that the sequencing of certain DNA fragments could be favored over others, causing a skew in the number of reads that is not correlated with nucleosome occupancy (Fig. 2).

Progress in Genome-Wide Nucleosome Mapping

Genome-scale maps of *in vivo* nucleosome positions. In 2004, the first two microarray studies examining nucleosome positions in *S. cerevisiae* were reported (16, 93). Limited by the microarray technologies available at the time, the resolution of those studies was relatively low. Despite this limitation, those authors were able to develop the first large-scale nucleosome maps and to identify a strong common nucleosome pattern around promoters, which often show a nucleosome-depleted region (NDR) surrounded by two highly positioned nucleosomes (16, 93). A pioneering study to map nucleosome positions at a nearly single-nucleosome resolution was carried out by Yuan et al. (207) in 2005. The development of microarrays with increased resolution made it possible to construct a microarray consisting of 50-bp oligonucleotide probes tiled every 20 bp, allowing those authors to examine 482 kb of the *S. cerevisiae* genome, including most of chromosome III and 223 additional regulatory regions. Those authors found that a large number of nucleosomes are "well positioned." This means that for a population of yeast cells, the nucleosome is present at the very same genomic location in every cell. In contrast, they found that only a minority of nucleosomes are delocalized or "fuzzy," occupying different locations in the genomes of the cells in a population. In addition, as in the earlier low-resolution studies, Yuan et al. (207) found that parts of promoter regions are often devoid of nucleosomes. These approximately 150-bp so-called "nucleosome-free regions" (NFRs) (also termed NDRs) are bordered by strongly positioned nucleosomes. Sequence analysis showed that the NFRs are evolutionarily conserved and often enriched in poly(dA:dT) sequences [homopolymeric runs of poly(A) or

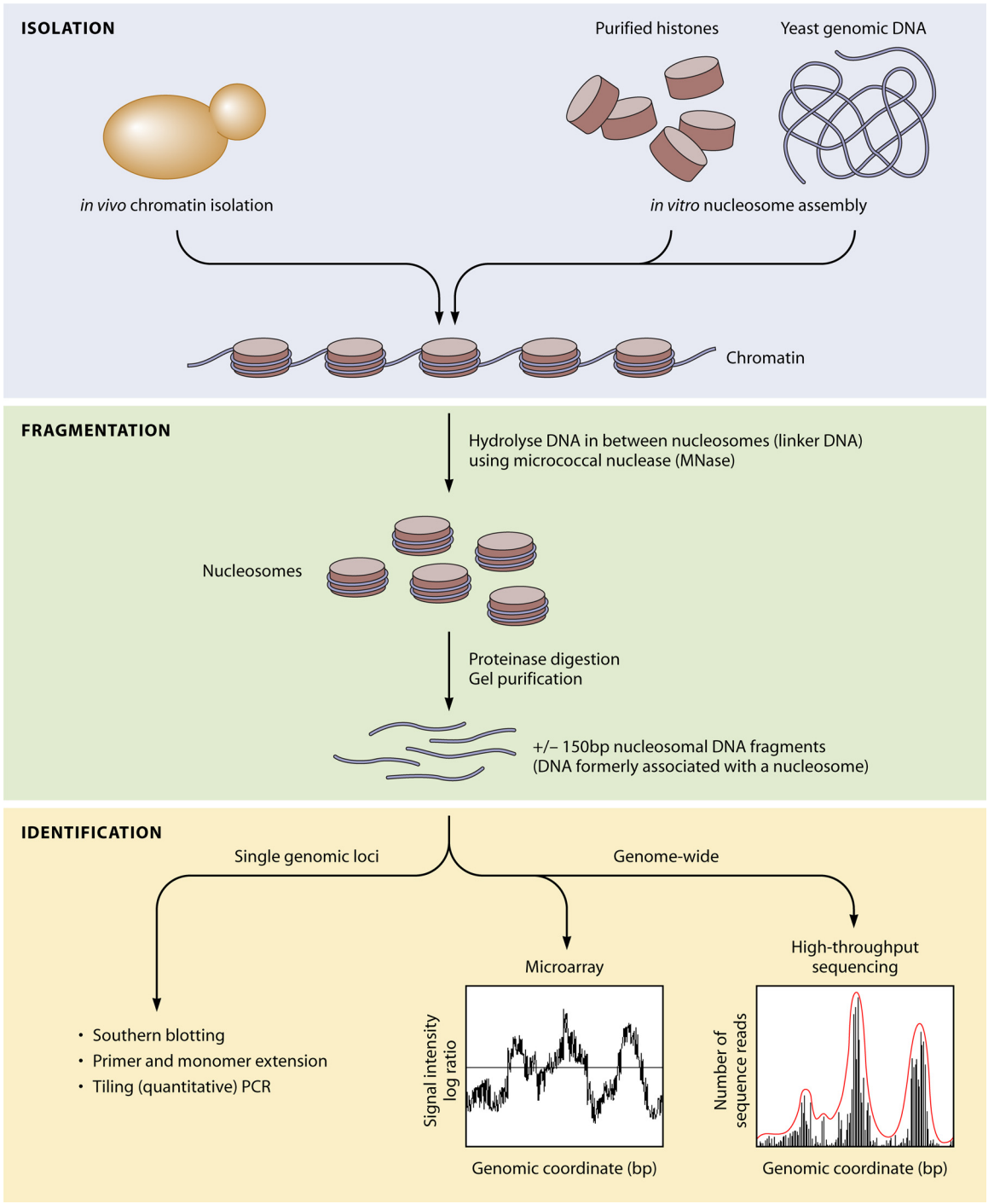

FIG. 2. Nucleosome-mapping technologies. *In vivo*-isolated or *in vitro*-reconstituted chromatin is fractionated by micrococcal nuclease (MNase) digestion, which hydrolyzes the linker DNA in between nucleosomes, generating single nucleosomes. After proteinase treatment, ~150-bp mononucleosomal DNA fragments are isolated by agarose gel electrophoresis. Subsequently, several techniques can be used to identify the collected DNA fragments and measure which fragments are enriched. Microarray hybridization and high-throughput sequencing allow the examination of nucleosome occupancy and positions on a genomic scale. Enriched fragments indicate that these sequences were integrated into a nucleosome, whereas depleted fragments were present in linker regions. See the text for details.

poly(T)] (see below for further details).

In 2007, the nucleosomes across the entire *S. cerevisiae* genome were mapped (96). Here, high-density tiling microarrays with 25-bp probes spaced every 4 bp were used, identifying over 70,000 positioned nucleosomes occupying 81% of the genome. That study confirmed the presence of intergenic NFRs on a genome-wide scale, present in promoters just upstream of the transcription start site (TSS).

The first study to use high-throughput sequencing technology to map nucleosomes focused on histone variant H2A.Z,

examining the incorporation of H2A.Z into nucleosomes across the genome (4). Here, chromatin was cross-linked and MNase digested as described above, followed by the immunoprecipitation of H2A.Z nucleosome core particles. The resulting pool of nucleosomal DNA was highly enriched for DNA incorporated into H2A.Z-containing nucleosomes, enabling the mapping of nucleosomes that contain the H2A.Z histone variant. That study showed that the orientation of the DNA helix on the histone surface (the so-called "rotational" position of a nucleosome) follows one predominant setting. As a consequence, when histone octamers form a nucleosome on the DNA, they select from several alternative positions that are separated from each other by the distance multiple of a helical turn so that the rotational angle of the histone core with respect to the DNA remains unchanged. Those authors also showed that nucleosomes take on various "translational" settings (i.e., the precise position of a nucleosome relative to a given DNA locus). In a subsequent study, that same group mapped global nucleosome positions using immunoprecipitation with antibodies against tagged histones H3 and H4 (109). More than 1 million reads of nucleosomal DNA were sequenced and mapped, confirming the presence of a 5′ NFR at 95% of all genes. In addition, those authors demonstrated the presence of a 3′ NFR at the ends of most genes (>95% of all genes), with a well-positioned nucleosome at the 3′ end of the coding region.

Around that same time, Shivaswamy et al. (159) used high-throughput sequencing to generate maps of nucleosome positions for yeast cells grown under different physiological conditions, comparing heat-shocked cells with control cells. Similar to the findings of Mavrich et al. (109), they reported the presence of NFRs at both ends of the coding region. That study did not reveal major shifts in nucleosome positioning between cells grown under normal conditions and stressed cells, although some specific nucleosomes were introduced, evicted, or moved in the populations grown under stress conditions. Here, nucleosome eviction generally correlated with gene induction. Both studies helped to establish a "statistical positioning" or "barrier" model for the organization of nucleosomes throughout the genome, which was first proposed by Kornberg and Stryer (discussed below) (83).

Another *in vivo* map of nucleosome positions in *S. cerevisiae* was compiled by Field et al. (47). Here, approximately 380,000 nucleosomes were sequenced and mapped to the yeast genome. Using a computational model, that study set out to determine nucleosome-positioning signals encoded in the DNA sequence. The model identified short DNA sequences (5-mer oligonucleotides) that correlate with a relative enrichment or depletion of nucleosomes. As was shown by previous genome-wide studies (109, 207), variants of poly(dA:dT) sequences were found to be the most dominant nucleosome-excluding DNA sequences, confirming that AT-rich sequences have a very low propensity to form nucleosomes (47) (see below).

Taken together, within a few years, multiple high-resolution genome-wide maps of *in vivo* nucleosome positions in *S. cerevisiae* were assembled. Jiang and Pugh (74) compared six of these nucleosome maps (47, 74, 96, 109, 159, 195). All of the maps were generated with laboratory strain S288C under similar growth conditions in rich medium (yeast-peptone-dextrose medium). While each of the maps was created by a different group using different technologies, the maps are nearly indistinguishable, with a median mapping error on the order of 5 to 7 bp genome wide. Using the data sets from all six studies, those authors compiled a consensus reference map of nucleosome positions in the yeast genome (74).

Genome-scale maps of *in vitro* nucleosome positions. All of the studies discussed so far focused on determining nucleosome positions *in vivo*. However, the affinity of DNA sequences for nucleosome formation is preferentially demonstrated *in vitro*, excluding the effects of *trans* factors and focusing solely on intrinsic DNA sequence preferences. In two recent studies, *in vitro* nucleosome maps were generated and compared with the *in vivo* nucleosome organization (79, 211). Using salt dialysis to reassemble chromatin from purified histone octamers and genomic DNA, Kaplan et al. (79) were able to construct a genome-wide map of *in vitro* nucleosome occupancy. They compared this map with the nucleosome maps constructed under three different growth conditions *in vivo*. The *in vitro* map shows many similarities to the *in vivo* maps, including nucleosome depletion at transcription factor (TF)-binding sites and transcription start and end sites and an agreement between the positions of highly localized nucleosomes. Overall, the maps showed a correlation of 0.74 for the nucleosome occupancy per base pair, indicating that nucleosome positions are encoded largely by intrinsic DNA sequence signals. However, the correlation of the maps is not uniform across the genome, and important differences include the lower level of depletion around transcription start sites *in vitro* than *in vivo* and the increased ordering of nucleosomes in coding regions observed with the *in vivo* map. These differences between the *in vivo* and *in vitro* maps indicate that apart from the intrinsic propensity for certain DNA sequences to form or deter nucleosomes, cellular components such as transcription factors, chromatin remodelers, and the transcription initiation machinery may also contribute to the chromatin organization *in vivo* (79).

In another study, Zhang et al. (211) purified both *S. cerevisiae* and *Escherichia coli* genomic DNAs and assembled them into chromatin by either salt dialysis or using the ACF assembly factor, an ATP-dependent chromatin assembly factor known to produce arrays of regularly spaced nucleosomes (50, 202). Those authors showed that yeast DNA has a higher intrinsic affinity for nucleosome formation, suggesting that the yeast genome has evolved to ease nucleosome formation. Similar to the data obtained by Kaplan et al. (79), the *in vitro* maps generated in that study showed regions at the transcription start and termination sites that intrinsically disfavor nucleosome incorporation. However, those authors noted that nucleosomes assembled *in vitro* show substantially less translational positioning (i.e., the position of a nucleosome relative to a given DNA locus) than observed *in vivo*, and they obtained a correlation coefficient of 0.54 between their *in vitro* and *in vivo* maps. Based on these observations, those authors concluded that intrinsic DNA sequence preferences are not a major determinant of *in vivo* nucleosome positions (211).

Thus, both studies reached opposite conclusions when assessing the relative importance of the DNA sequence in directing nucleosome organization. Technical and methodological issues concerning both studies have been the subject of an ongoing debate (78, 79, 136, 162, 210, 211). Whereas no con-

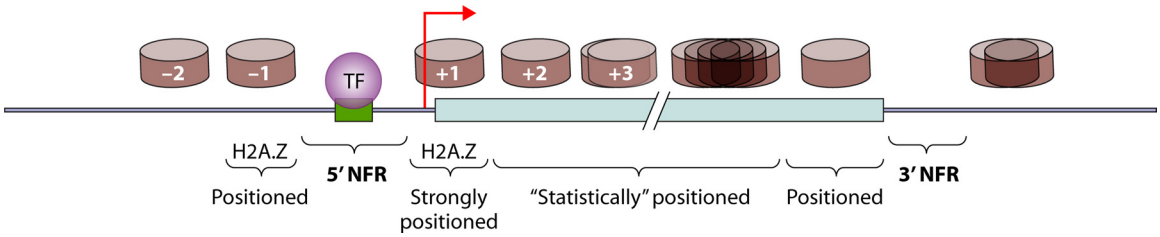

FIG. 3. Nucleosome architecture of the archetypical yeast gene. A 150-bp 5′ nucleosome-depleted region (5′ NFR) is surrounded by the highly localized and histone variant H2A.Z-enriched −1 and +1 nucleosomes. The 5′ NFR contains most functional *cis*-regulatory sequences, including transcription factor (TF)-binding sites. The +1 nucleosome is located just upstream of the transcription start site (TSS) (arrow). Downstream of the +1 nucleosome, each nucleosome is gradually less precisely localized than the previous upstream nucleosome. Beyond ~1 kb from the TSS, consensus spacing from the TSS dissipates. At the 3′ end, a positioned nucleosome precedes the 3′ NFR.

sensus has been reached, some differences might be explained by the different methods employed by both groups. In their study, Kaplan et al. (79) measured nucleosome density or occupancy, calculating the average amount of histones on a given region of DNA in a population. The nucleosome density is calculated by measuring the intensity of the signal when using a microarray or by counting the sequence reads when using high-throughput sequencing. For any given region, nucleosome occupancy directly reflects the intrinsic DNA-histone affinity. Hence, the data gathered by Kaplan et al. (79) offer good insights into the affinity of a short stretch of DNA for binding histone proteins.

However, the nucleosome occupancy does not necessarily offer detailed information about the exact position of a nucleosome, i.e., the precise position of an individual nucleosome with regard to a given DNA sequence. To determine the position of a nucleosome, one could, for example, calculate the position of the center of the nucleosome, and the standard deviation from this position would then be a measure of how well a nucleosome is positioned. A DNA sequence with a low nucleosome occupancy and, thus, a low intrinsic histone-DNA affinity might contain a highly positioned nucleosome, while the opposite can also be true. Thus, despite offering precise information about local histone-DNA affinities, nucleosome occupancy does not offer precise information about exact nucleosome positions. To calculate the contribution of histone-DNA interactions in positioning nucleosomes, one would have to calculate exact nucleosome positions, which is what Zhang et al. (211) did in their study. Based on their data, Zhang et al. (211) concluded that the correlation between the *in vivo* and *in vitro* maps is low, indicating that overall, DNA sequences have little impact on the exact position of a nucleosome, although notable exceptions occur, including poly(dA:dT) tracts (see below). Moreover, whereas the local DNA sequence might often not determine the precise position of a nucleosome, the local DNA sequence does influence local nucleosome occupancy.

Genome-Scale Maps Reveal a Common Chromatin Structure around Genes

The availability of genome-wide nucleosome maps and computational models has greatly improved our understanding of *in vivo* nucleosome organization. As discussed above, the different mapping studies have revealed that nucleosomes are highly organized, which is reflected by the fact that the majority of nucleosomes (about 80%) in *S. cerevisiae* are highly positioned or phased; i.e., they occur at the same (or at least nearly the same) location in virtually all the cells of a population (96, 207). In addition, these highly positioned nucleosomes are generally spaced at a set distance from each other, separated by short fragments of linker DNA. In *S. cerevisiae*, the linker DNA is on average 18 bp long, making the distance between adjacent nucleosome midpoints approximately 165 bp (147 bp of nucleosomal DNA and 18 bp of linker DNA) (96, 109, 159).

Genome-wide nucleosome maps also show that nucleosomes are organized onto yeast genes in common motifs, and specific patterns of nucleosome organization occur at both the 5′ and 3′ ends of genes (Fig. 3). Most yeast genes (about 95%) are characterized by a general depletion or even total absence of nucleosomes at their 5′ ends just upstream of the TSS (4, 74, 79, 109, 195, 207). This approximately 150-bp-long region is usually referred to as the 5′ nucleosome-free region (5′ NFR), although some authors have used the term "nucleosome-depleted region" (5′ NDR), because this region may be depleted of nucleosomes rather than completely nucleosome free (20, 62, 193). However, by constructing the first fully saturating depth-of-coverage nucleosome map, Jiang and Pugh (74) were unable to detect the presence of transient nucleosomes in the 5′ NFR, indicating that it may in fact be completely devoid of nucleosomes. Most functional *cis*-regulatory sequences, for example, TF-binding sites and TATA boxes, reside in the 5′ NFR, where the binding of *trans* factors is not obstructed by the presence of nucleosomes (163, 207) (Fig. 3).

The 5′ NFR is surrounded by two highly positioned nucleosomes, termed the −1 and +1 nucleosomes. The −1 nucleosome is located upstream of the NFR, usually within a region from −300 to −150 bp relative to the TSS. The downstream area of the NFR is bordered by the +1 nucleosome, on average the most strongly localized nucleosome in the yeast genome. The center of this nucleosome is located ~50 to 60 bp downstream of the TSS, and transcription starts ~10 bp into this +1 nucleosome (4, 93, 96, 109, 207). Both the −1 and +1 nucleosomes often contain the histone H2A variant H2A.Z, which facilitates transcription activation by histone loss (2, 4, 55, 98, 114, 137, 146, 209) (Fig. 3). Downstream of the strongly positioned +1 nucleosome, each nucleosome is gradually less precisely localized than the previous upstream nucleosome. Beyond ~1 kb from the TSS, consensus spacing from the TSS

dissipates, and there is an increased tendency for random nucleosome positions (109, 207) (Fig. 3).

Like the 5′ ends of genes, most 3′ ends (about 95%) are also characterized by a distinct nucleosome pattern. Here, an NFR with a positioned nucleosome immediately upstream has been identified (3′ NFR) (109, 159). The location of the 3′ NFR coincides with the region where RNA polymerase II (Pol II) terminates transcription (Fig. 3). The function of the 3′ NFR remains to be studied in detail, but it has been noted that it is often bound by the general transcription factor TFIIB, which has been implicated in gene looping. In this form of gene regulation, the 5′ and 3′ ends of genes appear to interact, possibly enabling the recycling of the transcription machinery after each round of transcription. Interestingly, it has been observed that many antisense transcripts initiate in this region (30, 109).

CIS DETERMINANTS OF NUCLEOSOME POSITIONING

Predicting Nucleosome Positions from Local DNA Sequence

Genome-wide maps provide a wealth of information about the underlying factors that affect nucleosome positioning. More specifically, genome-scale positions of nucleosomes can be analyzed to explore if there is any correlation with local (*cis*) factors, most notably DNA sequences. A quick glance at nucleosome positioning maps reveals that there does not seem to be an obvious "signature" DNA sequence that is always found within a nucleosome or a linker region. Hence, a more detailed analysis is needed to uncover possible correlations between the DNA sequence and nucleosome positioning. One efficient way to do this is to develop algorithms that predict nucleosome positioning based on local DNA sequence information. The predictive capacity of such models offers a good estimation of the degree to which nucleosome positions are determined by *cis* factors, such as the DNA sequence, as well as derived parameters, such as the physical properties of the local DNA helix.

Predicting nucleosome positioning based on physical DNA properties. Several research groups have tried to predict *in vivo* and *in vitro* nucleosome positions in yeast and other organisms using computational models based on properties of the underlying DNA sequence. Physical models often explain observed sequence patterns in terms of elastic energies associated with DNA bending. When a nucleosome is formed, DNA wraps very tightly around the histone octamer. The free energy of the bending of the DNA is sequence dependent, with flexible sequences bending more easily than stiff ones. For each dinucleotide, the free energy is calculated based on structural DNA data, for example, crystal and nuclear magnetic resonance (NMR) structures of nucleosomes. The advantage of physical models for nucleosome positioning is that they are biologically meaningful: they explain why a certain sequence forms or does not form a nucleosome. However, the accuracy of these models depends greatly on the quality of the data about the physical properties of DNA loci and the exact influence that these properties have on nucleosome formation. Several studies reported physical models; however, their genome-wide accuracy has not yet been thoroughly investigated (111, 118, 152, 178, 182).

Predicting nucleosome positioning based on the local DNA sequence. A second, broad group of models is based on the (high-throughput) nucleosome positioning data discussed above. The general strategy used is to identify nucleosome-favoring and nucleosome-excluding DNA sequence motifs and then use these sequences as a training set in machine learning algorithms to predict common sequence patterns in nucleosomal DNA. The identified motifs are subsequently used to predict nucleosome positions genome wide. Hence, in contrast to physical models, these sequence-based models do not start from a hypothesis about factors that may affect nucleosome formation. Instead, they are "black-box" models that identify merely correlations between the DNA sequence and nucleosome density. However, once clear correlations are found, it is often possible to speculate about the underlying biological reason for the observed correlation.

Two early bioinformatics models were based on limited sequence information, each using different sets of approximately 200 DNA sequences from well-positioned nucleosomes as training sets to compute common nucleosome-favoring sequence patterns. Ioshikhes et al. (70, 71) obtained a nucleosome-positioning sequence (NPS) of AA/TT dinucleotides occurring every 10 bp. Next, they searched for correlations of the NPS with the rest of the genome, identifying nucleosome-depleted regions in most yeast promoters (strongest anticorrelation with the NPS) and a well-positioned nucleosome just downstream of this region (strongest correlation with the NPS). A difference in the correlation pattern was observed for TATA-less and TATA-containing genes, with TATA-less genes showing the strongest NPS/anti-NPS/NPS motif (71). Similarly, another training set containing 199 yeast DNA sequences from well-positioned nucleosomes was used by Segal et al. (154). An analysis of dinucleotide distributions revealed a 10-bp periodicity of AA/TT/TAs, which is offset every 5 bp with a 10-bp periodicity of GC.

Overall, the predictive power of these "first-generation" models is relatively poor; for example, the model developed by Segal et al. (154) can predict about 50% of the *in vivo* nucleosome organization. Segal and coworkers further refined their model by incorporating not only nucleosome-favoring sequences but also nucleosome-disfavoring (so-called anti-nucleosomal) sequences. They achieved this by including 5-mer motifs that might function as nucleosome-favoring or -disfavoring signals (47). Furthermore, those authors expanded their training set to approximately 380,000 yeast nucleosomal DNA sequences. This significantly improved model identified poly(dA:dT) tracts as major determinants of nucleosome organization by acting as nucleosome-excluding sequences. Recently, a version of this model trained on *in vitro* data was reported (79) (discussed above), and in another study, the model was expanded to include interactions between adjacent nucleosomes (104). Compared with the "first-generation" models, the predictive power of these refined models has increased dramatically. For example, the model trained on *in vitro* data (79) shows correlations with the *in vitro* and *in vivo* maps of 0.89 and 0.75, respectively.

Other bioinformatics studies have used different approaches. Peckham et al. (130) employed a support vector machine (SVM) classifier incorporating the experimental data set of nucleosome positions reported by Yuan et al. (207). The

strongest and weakest nucleosome-forming 50-bp fragments were converted into vectors of *k*-mer frequencies ($k = 1$ to 6). As in other studies, those authors found that AT-rich *k*-mer oligonucleotides disfavored histone binding, whereas GC-rich sequences enhanced nucleosome formation. Those authors therefore argued that the AT or GC content is the most important factor determining the affinity of a DNA sequence for binding histone proteins (130). Yuan and Liu (206) also developed an algorithm that distinguishes histone-bound sequences from linker DNA. Their method detects periodic patterns in the sequences and calculates the presence of dinucleotide signals.

Several studies have used hidden Markov models (HMMs) to calculate nucleosome occupancies directly from the log intensity data obtained from microarray-based nucleosome positioning experiments (96, 207, 208). Lee et al. (96) used an HMM to examine their *in vivo* nucleosome positioning data for dinucleotide signals. Those authors calculated that the AA/TT/TA dinucleotide signal was not highly correlated with global nucleosome occupancy, but as with the other models, they also observed a good correlation between GC content and nucleosome positions (96). More recently, Tillo and Hughes (169) set out to determine which specific DNA sequence properties are most important for intrinsic nucleosome-forming preferences. The *in vitro* data set reported by Kaplan et al. (79) was used, and a large number of straightforward candidate features was selected, such as %GC content. Next, a linear regression algorithm was used to create a linear combination model. The resulting model has only 14 parameters and predicts the *in vitro* nucleosome occupancy data with great accuracy ($R = 0.86$). For comparison, the model reported by Kaplan et al. (79) includes dinucleotide frequencies and all possible 5-mer oligonucleotides (see above), resulting in more than 2,000 parameters, and performs only slightly better ($R = 0.89$). A close examination of the most important features of this model indicates that %GC contents and poly(A) runs are the two dominant factors, with a model based on the %GC content alone showing a correlation of 0.71 with the *in vitro* data. As there are no dinucleotide periodicities in this model, nucleosome exclusion by poly(A) and related sequences appears to be the dominant feature of *in vitro* nucleosome reconstitution assays (169).

AT Content Is a Major *cis* Factor Influencing Nucleosome Positions

Evidence that AT content influences nucleosome positioning. Nucleosome maps and computational models shed light on the nature of sequence-dependent positional signals that help to direct the chromatin structure. By simply comparing the common features of DNA sequences that seem to act as nucleosome-favoring or -disfavoring sequences, one obvious trend emerged. The AT content is a good predictor of nucleosome occupancy, and a high %AT content correlates with low nucleosome occupancy (67, 169). Most typically, one or more homopolymeric runs of poly(A) or poly(T), referred to as poly(dA:dT) sequences, occur in the 5′ and 3′ NFRs, where they act as nucleosome-excluding sequences (9, 47, 72, 96, 109, 155, 163, 207). In addition, a significant proportion (about 20%) of yeast promoters contains stretches of tandem repeats that are often extremely AT rich and act as nucleosome-disfavoring sequences that help to establish the 5′ NFR in these promoters (188). Because these repeats are unstable and highly variable, they help to establish variable nucleosome-free DNA structures that can influence nucleosome positioning and, thus, gene expression in nearby regions (188) (see below).

How do AT-rich DNA sequences influence nucleosome positioning? It is believed that AT-rich tracts deter nucleosomes because these sequences are unusually stiff, thereby resisting the sharp bending required for histone binding. However, this presumed enhanced stiffness has not been proven directly, and data from recent studies do not support a high intrinsic stiffness of AA dinucleotides (35, 52, 124, 155). Poly(dA:dT) tracts are known to have a different structure, with a shorter helical structure and a narrow minor groove, which may contribute to their low propensity to form nucleosomes (32, 119, 129, 143). In addition, data from crystallography studies suggest the presence of bifurcated hydrogen bonds between an A base and two T's on the opposite strand (32, 119, 201). However, the existence of these H bonds and their contribution to the structure of poly(dA:dT) remains unclear. Finally, several experiments showed that the unusual structure of poly(dA:dT) tracts is length dependent, and tracts of lengths of 4 bp or greater have been shown to adopt a special cooperative state that could disfavor inclusion into nucleosomes (9, 153). In summary, evidence suggests that poly(dA:dT) tracts have structural, dynamic, and/or mechanical properties that differ from generic sequences and prevent incorporation into nucleosomes (155).

Antinucleosomal sequences and the barrier model for nucleosome positioning. Antinucleosomal AT-rich sequences resist nucleosome formation. Throughout the genome they play an even larger role by acting as boundaries against which nucleosomes are positioned (109). Indeed, NFRs established by poly(dA:dT) tracts tend to be surrounded by well-positioned nucleosomes (109, 207). Especially at the 5′ NFR, the +1 nucleosome and, to a lesser extent, the −1 nucleosome form barriers that can direct the positioning of other nucleosomes as far as approximately 1 kb away. Beyond 1 kb, nucleosomes are less localized and well positioned, and MNase digestion patterns indicate that the histones might be more loosely associated with the DNA. Thus, antinucleosomal sequences such as poly(dA:dT) tracts create a "barrier" that favors the formation of highly positioned nucleosomes directly adjacent to the antinucleosomal sequence, which in turn direct the positions of neighboring nucleosomes (109). This model for the sequence-directed organization of nucleosomes throughout the genome is called the "statistical positioning" or "barrier" model (83, 109) (Fig. 3).

While proposed and experimentally verified more than 2 decades ago (45, 83), the barrier model has only recently been applied to the nucleosome organization around nucleosome-depleted promoter regions (109, 207). In several recent studies, the barrier model for nucleosome organization surrounding 5′ NFRs has been confirmed (21, 112, 115, 183). In their study, Möbius and Gerland (115) applied a minimal physical model to quantitatively describe the nucleosome density upstream of the −1 nucleosome and downstream of the +1 nucleosome. They found that statistically positioned nucleosomes in these regions behave according to the "Tonks gas" model, with density oscillations occurring close to the bound-

aries at dense packing (115). In another study, Vaillant et al. (183) ranked intragenic regions by the distance between their first and last nucleosomes and confirmed that the nucleosome organization of genes is a consequence of statistical ordering induced by inhibitory boundaries at both sides of genes (183). Finally, an experimental study combining atomic force microscopy with physical modeling showed that boundaries are formed by sequences that encode high-energy barriers. These sequences impair nucleosome formation and direct the positions of the surrounding nucleosomes according to statistical ordering principles (112).

Nucleosome-favoring sequences. Apart from poly(dA:dT) tracts, which act as nucleosome-deterring sequences, some sequence patterns seem to promote nucleosome binding. The large-scale studies and models discussed above showed that A/T dinucleotides spaced at 10-bp intervals occur throughout nucleosomes. In addition, this pattern is offset by 5 bp with 10-bp periodicities of G/C dinucleotides (4, 47, 71, 109, 111, 130, 149, 154, 179, 190, 199). AT-rich dinucleotides have specific deformation properties, favoring DNA bending in a specific direction, expanding the major groove. The appearance of the dinucleotides at every 10 bp may align the bends in the same direction. Similarly, G/C dinucleotides also confer bending, contracting the major groove. The alternations of A/T and G/C dinucleotides could therefore affect the DNA helix in such a way that the energy required to bend the DNA is greatly reduced, making these sequences thermodynamically favorable as binding sites for histones (75). Dinucleotide periodicities likely direct the overall rotational setting of a nucleosome (i.e., the orientation of the DNA helix on the histone surface) while having little effect on determining the exact translational position (i.e., the position of a nucleosome relative to a given DNA locus), consistent with the barrier model for nucleosome positioning (75, 109).

In conclusion, it has become clear that the AT content and, in particular, nucleosome exclusion by AT-rich sequences play a crucial role in directing chromatin organization in *S. cerevisiae*. By creating NFRs, AT-rich sequences form barriers that position the surrounding nucleosomes according to the principles of statistical positioning. In addition, dinucleotide periodicities increase the affinity of DNA sequences for nucleosome formation and direct the rotational positioning of nucleosomes.

TRANS DETERMINANTS OF NUCLEOSOME POSITIONING

So far, we have focused on sequence-driven determinants of nucleosome positions. However, as discussed above, it is clear that the *in vivo* nucleosome organization cannot be explained by DNA sequence preferences alone. Other factors contribute to the positioning of nucleosomes, including RNA polymerases, chromatin remodeling complexes, transcription factors, histone variants, and posttranslational histone modifications.

Influence of RNA Polymerase on Nucleosome Positioning

RNA polymerases have a major impact on the *in vivo* nucleosome structure. While they move along the DNA, they encounter nucleosomes that form a barrier for transcription. The passage of RNA polymerase requires the (partial) disruption of DNA-histone contacts, resulting in nucleosome eviction and/or sliding (34, 73, 151, 164, 165, 192). Because RNA polymerases are so widespread, they might very well be the most important of all *trans* factors affecting nucleosome positions.

Several recent genome-wide studies showed that Pol II (transcription) affects the nucleosome structures of the promoter, the terminator, as well as coding regions. In promoters, the −1 nucleosome is evicted in the presence of Pol II, and in agreement with this observation, the NFR width appears to correlate with transcription levels (81, 159, 187, 193). At terminators, the depletion of Pol II as well as lower transcription levels cause an increase in nucleosome occupancy, indicating that the formation of the 3′ NFR is mediated primarily by transcription-based mechanisms (42).

Transcription rates also affect nucleosomes that are positioned over coding regions. Here, nucleosomes are more delocalized and less dense at higher transcription rates, with the possible exception of the +1 nucleosome (81, 159). In addition, it has been shown that the loss of Pol II causes nucleosomes to move away from the NFR, pointing toward a role for RNA polymerase in nucleosome sliding. These shifts in the absence of Pol II are small (about 10 bp) but more dramatic in very highly expressed genes, such as ribosomal protein and amino acid metabolism genes (193). A comparison with the *in vitro* data obtained by Kaplan et al. (79) showed that nucleosome eviction and sliding by Pol II in promoter and coding regions antagonize the thermodynamically preferred nucleosome positions (79, 193). Thus, Pol II plays an active role in shaping the chromatin structure of the transcribed genome.

How does transcription elongation by Pol II through chromatin proceed? *In vitro*, Pol II is capable of passing by a nucleosome by releasing just one H2A/H2B dimer (64, 164, 165). The remaining histone hexamer survives Pol II passage. Based on this observation, a mechanism was proposed in which the first step is the partial displacement of the histone hexamer from the DNA. Next, a small intranucleosomal DNA loop is formed when the released hexamer surface rebinds to the DNA behind Pol II. In a final step, the histone hexamer is recovered behind Pol II, resulting in a minimal exchange of H3 and H4 histones throughout transcription (86). At low and moderate rates of transcription, the H2A/H2B dimer rebinds the histone hexamer after Pol II passage. However, at high transcription rates, the histone hexamer may also be evicted by subsequent transcribing Pol II complexes, resulting in the removal of all core histones from the DNA (87). The chromatin structure recovers after the transcription rate decreases.

Chromatin Remodeling Complexes

Chromatin remodelers are ATP-dependent multiprotein complexes that use the energy of ATP hydrolysis to move, eject, or restructure nucleosomes (25). *In vitro*, these complexes have been shown to control the spacing between nucleosomes on long stretches of DNA (50). In addition, they can drive nucleosomes to less favorable locations on short DNA fragments *in vitro* (144, 202).

There are several classes of chromatin remodeling complexes, including the SWI/SNF (switch/sucrose-nonferment-

able), ISWI (imitation switch), INO80 (inositol-requiring), and CHD (chromo-helicase/ATPase-DNA-binding) families. All complexes possess a conserved ATPase subunit that provides free energy to facilitate DNA translocation, but different subfamilies exhibit divergent remodeling activities (25).

The RSC complex. The RSC complex is a chromatin remodeling complex from the SWI/SNF family that binds about 700 targets in the yeast genome. RSC targets are mostly Pol III-transcribed genes and a subset of Pol II promoters, many of which carry a specific DNA-binding motif for the Rsc3 subunit (13, 29, 121). Conditional mutants of the catalytic subunit Sth1 display an increased nucleosome occupancy over Pol III genes (128). In addition, the depletion of Sth1 causes the shrinking of the NFR in RSC-occupied promoters, accompanied by the movement of the flanking nucleosomes (13, 59, 128). This is often the case for promoters containing TATA boxes. Here, the TATA box is usually located inside the −1 nucleosome, and chromatin remodelers are required to expose the TATA box (15). The mechanisms for the increase in nucleosome occupancy appear to vary and consist of a combination of nucleosome sliding as well as binding by new nucleosomes.

The Swi/Snf complex. The SWI/SNF enzymes can promote nucleosome repositioning, alternative nucleosome structures containing DNA loops, and nucleosomes with altered histone compositions (25). For example, the SWI/SNF complex stimulates the binding of the Gal4 activator to nucleosomal DNA *in vitro* (28, 89) and can facilitate Gal4 binding to occluded sites to promote transcription *in vivo* (19) by removing nucleosomes that occupy the Gal4-binding site.

The ISWI family of chromatin remodelers. Isw2 is a chromatin remodeler from the ISWI family that moves nucleosomes from one place to another laterally. Whitehouse and Tsukiyama (196) showed that at the *POT1* promoter, Isw2 functions by moving a nucleosome from its sequence-directed position to a neighboring, less favorable location (196). Subsequently, genome-wide Isw2 targets were mapped by identifying differences between wild-type and Δ*isw2* mutant strains. More than 1,000 regions showed a disrupted nucleosome structure in the Δ*isw2* mutant strain compared to the wild-type strain, among which were about 12% of all yeast promoters. Here, it was shown that Isw2 functions by moving nucleosomes into 5′ and 3′ NFRs, thereby overriding intrinsic sequence-based nucleosome positioning signals like poly(dA:dT) sequences. The inactivation of the Isw2 complex leads to an average shift of about 15 bp in the location of these nucleosomes relative to that of wild-type cells. By doing so, transcription initiation and termination sites become incorporated into nucleosomes, preventing transcription from suppressed genes and antisense transcription (195).

The Isw2 homologue Isw1 has also been shown to affect nucleosome positions. At the *MET16* promoter, the −1 and +1 nucleosomes are repositioned by Isw1 upon activation to allow the promoter to be accessed by the transcription machinery (117). Besides its role in transcriptional activation, Isw1 has also been implicated in transcriptional elongation (117, 185). Tirosh et al. (176) examined the genome-wide effect of Isw1 on nucleosome positioning and found that the deletion of *ISW1* affected the positions of nucleosomes within coding regions in about half of all genes. Specifically, the positions of nucleosomes in mid-coding regions were shifted toward the 5′ ends of genes. These shifts were correlated with the presence of H3K79 trimethylation and were enriched at genes with cryptic initiation sites. These data suggest that Isw1 maintains a repressive chromatin structure during transcriptional elongation through its interaction with specific histone marks.

Together, chromatin remodeling complexes allow promoters to adopt different nucleosome distributions, exposing or occluding different TF-binding sites and promoting TSS access. Thus, nucleosome sliding, eviction, and/or remodeling are important mechanisms for regulating nucleosome positioning and gene activation.

Transcription Factors

In vivo nucleosome positions can be influenced directly by TF binding. TFs recognize and bind short DNA motifs, thereby wrapping around the DNA or inducing a specific DNA conformation. As a consequence, TF-bound DNA may no longer be prone to form a nucleosome, and conversely, TFs might not bind nucleosomal DNA. Thus, nucleosomes and TFs compete for access to the DNA, which is a major mechanism by which nucleosomes influence transcriptional activity.

Genome-wide measurements of *in vivo* nucleosome positions are consistent with this model. Many of the bound TF-binding sites reside in NFRs where accessibility is increased due to the low nucleosome occupancy, making it thermodynamically more favorable for TFs to bind (4, 54, 96, 207). The *in vitro* reconstitution of the yeast genome into nucleosomes revealed that part of the nucleosome depletion observed at TF-binding sites is intrinsically encoded in the genome, through the DNA sequence preferences of nucleosomes. Poly-(dA:dT) sequences, for example, disfavor nucleosome occupancy over the tract but also over the adjacent DNA regions, including TF-binding sites that are located very close to the poly(dA:dT) tract (79). However, several binding sites are more depleted of nucleosomes *in vivo* than *in vitro* (47, 79, 211). In these cases, TF binding likely creates or extends an NFR. Examples include the multifunctional transcription factors Abf1, Rap1, and Reb1. Here, it was shown that the NFRs of promoters that have an Abf1-, Rap1-, or Reb1-binding site have increased nucleosome occupancy when the relevant TF is depleted from the cell (47, 59, 79).

The mechanism of nucleosome depletion by multifunctional transcription factors is not always clear. The transcription factor Abf1 can expel a well-positioned nucleosome *in vitro* (204). It does not have an ATPase domain, so it is unlikely that it can actively remove or slide a nucleosome from its binding site. Most likely, binding-site exposure through (partial) nucleosome unwrapping allows Abf1 access to its binding site (8, 10, 131). This is consistent with the observation that many TF-binding sites are located near the entry and exit sites of nucleosomes, where DNA unwrapping originates (4, 9, 10, 134, 195). Consequently, Abf1 competes directly with the already-present nucleosome for binding. The outcome of this competition will depend on the affinity of Abf1 for its binding site, the affinity of the histone octamer for the DNA sequence, and the relative concentrations of both Abf1 and nucleosomes (156). The relatively high abundance of the Abf1 protein could explain why Abf1 induces the formation of NFRs, whereas other (less abundant) TFs do not (23, 120). The binding of Abf1 might

stabilize the nucleosome depletion and facilitate the binding of other TFs (3, 127).

Rap1 has been implicated as both an activator and a repressor of some of the most highly and lowly expressed genes in the cell, by directing nucleosome disruption or nucleosome formation, respectively (51, 53, 88, 161, 205). These apparently opposing functions could be linked to the location of Rap1 and nucleosomes in promoter regions. The −1 nucleosome is selectively bound by Rap1, and binding is enriched at divergent promoters, where both genes share the same −1 nucleosome (81). Rap1 binds the rotationally exposed first and second major grooves of DNA inside the nucleosome border (145), potentially imposing phasing (i.e., positioning the nucleosomes in the same way relative to the nucleotide sequence in all cells) onto the −1 nucleosome and/or repositioning this nucleosome over a very short distance. Indeed, Rap1-bound nucleosomes show increased phasing compared to other −1 nucleosomes (81). At certain genes, Rap1 promotes nucleosome displacement or even eviction. In the case of the highly expressed ribosomal protein genes, the −1 nucleosome is usually not present (81).

Reb1 is believed to bind promoter regions and promote NFR formation at certain sites (11, 40, 59, 116, 137, 142). Koerber et al. (81) showed that Reb1, like Rap1, strongly favors the −1 nucleosome of divergently transcribed genes. However, Reb1-binding sites are strongly enriched at the NFR-proximal border of the −1 nucleosome, indicating that Reb1 promotes NFR formation by creating a boundary (81). Consistent with this, genes that had Reb1-bound nucleosomes had smaller NFRs in a Reb1-depleted strain (13, 81).

Interestingly, Hartley and Madhani (59) recently showed that promoters regulated by the ATP-dependent chromatin remodeling RSC complex also showed increased nucleosome occupancy in a conditional RSC mutant. Abf1- and Reb1-regulated promoters form a subset of the RSC-regulated promoters, suggesting that Abf1 and Reb1 might evict nucleosomes *in vivo* by recruiting the RSC complex (59).

Posttranslational Histone Modifications and Histone Variants

The canonical histone proteins H2A, H2B, H3, and H4 can undergo extensive posttranslational histone modifications, mostly but not exclusively in their histone tails, which alter their interactions with DNA and nuclear proteins. The complete removal of the histone tails often results in small (about 10- to 20-bp) positional changes, especially for those nucleosomes that are not positioned over strong nucleosome-favoring sequences (56, 203). Histone tail removal as well as tail hyperacetylation also result in an increased accessibility of nucleosomal DNA (7, 133) and more unstable nucleosomes (197). However, it is likely that the indirect effects of posttranslational histone modifications on nucleosome positioning are more substantial, for instance, by influencing protein-protein interactions and recruiting ATP-dependent chromatin remodeling complexes. The activity of SWI/SNF, for example, can be enhanced by histone acetylation (60, 166). Not only does acetylation provide acetyl-lysine-binding sites for SWI/SNF (61), it also neutralizes positively charged lysines (33). The latter decreases the electrostatic affinity between the negatively charged phosphate groups of the DNA and the positively charged histone residues. As a consequence, higher-order repressive chromatin structures might be disrupted (191).

Apart from histone modifications, core histones can also be replaced by histone variants which have a (slightly) different amino acid sequence. Histone variants usually occur in specific chromosomal regions; for example, the well-studied H2A variant H2A.Z is found predominantly at the borders of the 5′ NFR (55, 137, 209). Nucleosomes containing histone variants might have altered bonds and interactions with the DNA, and it remains to be studied whether this affects the positioning of nucleosomes along the DNA polymer. In the case of H2A.Z, differences in the histone tail as well as several changes in internal residues might affect interactions of H2A.Z with itself and the H3/H4 tetramer, resulting in a less stable nucleosome (55, 137, 209).

INFLUENCE OF NEIGHBORING NUCLEOSOMES AND HIGHER-ORDER CHROMATIN STRUCTURES

A third major factor that influences local nucleosome positioning is the effect of neighboring nucleosomes and higher-order DNA structures. Such "steric" and "structural" effects can act locally (i.e., in *cis*) but also over longer distances (i.e., in *trans*).

Nucleosomes as Beads on a String

Since nucleosomes cannot overlap, the position of one nucleosome limits and directs the position of adjacent nucleosomes, much like beads on a string. Genome-wide maps of nucleosome positions have shown that nucleosomes in general occur at fixed distances from each other. In *S. cerevisiae*, the experimentally determined average linker length is approximately 18 bp (96, 109, 159), and longer average linker lengths occur in multicellular organisms (14, 110, 150, 184). Analyses of nucleosomal arrays and dinucleotide sequences have confirmed that linkers have preferred lengths, and steric exclusion is one important factor determining the space between consecutive nucleosomes (27, 189, 198). In addition, linker length is also determined by histone H1, a small histone protein which is not part of the histone octamer but instead specifically binds linker DNA. A partial H1 depletion results in shorter linker lengths and higher nucleosome densities (156). Since nucleosomes occur at fixed distances from each other, this would imply that if one nucleosome is highly positioned [for example, because of an antinucleosomal poly(dA:dT) tract], this would also determine the positions of the adjacent nucleosomes, as described previously by the barrier model for nucleosome positioning (83, 109). In fact, this principle of the long-range effect of the steric hindrance of neighboring nucleosomes is implicitly incorporated into the barrier model for nucleosome positioning (see above).

Neighboring nucleosomes have also been shown to interact with each other, both in one-dimensional nucleosomal arrays as well as in higher-order chromatin structures. The histone tails appear to play an important role in mediating these internucleosomal interactions (12, 17). The importance of interactions between adjacent nucleosomes in positioning nucleosomes was recently confirmed by a model predicting the

nucleosome structure. Lubliner and Segal (104) reported a computational model that takes into account the interactions between neighboring nucleosomes. They found that this model performs better at predicting both *in vitro* and *in vivo* nucleosome positions than previous models that assumed that the association of a nucleosome to DNA at one place is independent of the associations of nucleosomes elsewhere (104).

Engeholm et al. (39) used an *in vitro* dinucleosomal model system to show that adjacent nucleosomes can collide with each other. Those researchers found that when a nucleosome moves toward a neighbor, DNA at the interface is relaxed and stabilized, perhaps by a mechanism similar to that employed by TFs when binding to sequences on the edge of nucleosomes. Next, one nucleosome can invade the DNA territory of another. These collision events would cause the DNA template of nucleosomes to (temporarily) overlap (39).

Our recent study (A. Jansen and K. J. Verstrepen, submitted for publication) further demonstrates the importance of surrounding (distal) nucleosomes for local nucleosome patterning. In this study, a series of truncated versions of the *URA3* gene and its promoter is inserted into various locations in the *S. cerevisiae* genome. It was found that as long as the native poly(dA:dT) tract in the *URA3* promoter is present, the nucleosome positioning in the *URA3* promoter and open reading frame (ORF) is virtually independent of the surrounding nucleosome positions. However, when the poly(dA:dT) barrier is removed, nucleosome patterning at the *URA3* gene becomes dependent on the nucleosome positions of the surrounding insertion site. These experiments demonstrate that well-positioned distal nucleosomes (for example, nucleosomes that border an antinucleosomal barrier element) play an important role in positioning neighboring nucleosomes, thereby confirming this central pillar of the barrier model for nucleosome positioning (Jansen and Verstrepen, submitted).

Influence of Higher-Order Chromatin Structure

The one-dimensional beads-on-a-string nucleosome organization cannot be seen independently from the higher-order chromatin structure. Nucleosomes are spatially close to their upstream and downstream neighbors but also to other nucleosomes that are adjacent in three-dimensional space because of higher-order packaging (Fig. 1). Packing constraints from the higher-order folding of the chromatin fiber could therefore enforce nucleosome positioning (102). Attractive and repulsive interactions between nucleosomes in an array will be determined in part by interactions imposed by the structure of the chromatin fiber. Previous studies have shown the existence of direct electrostatic interactions between spatially closely positioned nucleosomes (22, 36, 106). The modification or substitution of amino acids in the histone tails or the introduction of histone variants alter the nucleosome surface and can change these interactions (1, 41, 66, 103, 160). For example, attractive nucleosome-nucleosome interactions mediated by N-terminal histone tails and dependent upon the acetylation status of lysine 16 in the histone H4 N-terminal domain have been shown to exist and could affect nucleosome positions (160). However, these interactions in three-dimensional space remain difficult to predict and interpret, making it extremely challenging to incorporate this information into predictive models of nucleosome structure.

INFLUENCE OF NUCLEOSOME POSITIONING ON GENE REGULATION

The chromatin structure has a major impact on virtually all cellular processes involving DNA. The most notable example is transcriptional regulation. For example, as described above, nucleosomes and TFs are believed to compete for binding to the same DNA sequence. Here, we briefly summarize how nucleosome positions affect transcription and how cells can exploit this to achieve a tailored, fine-tuned regulation of specific genes.

Nucleosome Positions and Gene Expression

A number of studies of yeast have shown that nucleosome depletion causes derepression in genes, even when their transcriptional activators are not present (38, 57, 58, 63). Thus, nucleosomes in promoters appear to function as nonspecific repressors of transcription.

However, nucleosomes also play a more specific role in regulating the activation and repression of transcription. Studies have shown that sequences to which a TF can bind are far more prevalent than sites that are actually bound by a factor *in vivo* (99, 100). This may be explained by the presence of positioned nucleosomes that prevent the binding of a TF to a TF-binding site that is not "intended" to be bound, for example, sites without a biological function that occur randomly in the genome. Indeed, as discussed above, many (functional) binding sites are located within NFRs, where they are readily accessible for TF binding (93, 96, 109, 159, 207).

It is important that, because of their dynamic nature, nucleosomes can also control gene expression in a nonstatic manner, for example, by transiently blocking biologically relevant TF-binding sites or by transiently allowing access to such a site. This is often facilitated by the actions of chromatin remodeling factors (97, 200). For example, in the promoters of *PHO5*, *GAL1*, *CUP1*, and *SUC2*, nucleosome loss upon transcriptional activation is facilitated by remodeling complexes. Conversely, nucleosomes are assembled or stabilized on these promoters during transcriptional repression (6, 44, 80, 158).

In the case of the *PHO5* promoter, nucleosomes also help to establish the precise response of the promoter to different levels of phosphate starvation. Multiple binding sites for the transcription factor Pho4 occur in both nucleosome-occupied and nucleosome-free regions of the *PHO5* promoter (186) (Fig. 4). The accessible Pho4-binding sites in the linker regions set the threshold of phosphate starvation for the Pho4-mediated activation of *PHO5*. However, once the threshold for *PHO5* activation is reached, the total number and strength of all the Pho4-binding sites, including sites in nucleosome-occupied regions, determine the maximal level of *PHO5* transcription (90). This is because the recruitment of an ATP-dependent remodeling factor evicts nucleosomes, thereby exposing the Pho4-binding sites previously occluded by nucleosomes (Fig. 4).

Whereas some studies showed the dynamic nature of the chromatin-dependent regulation of specific genes, it is unclear

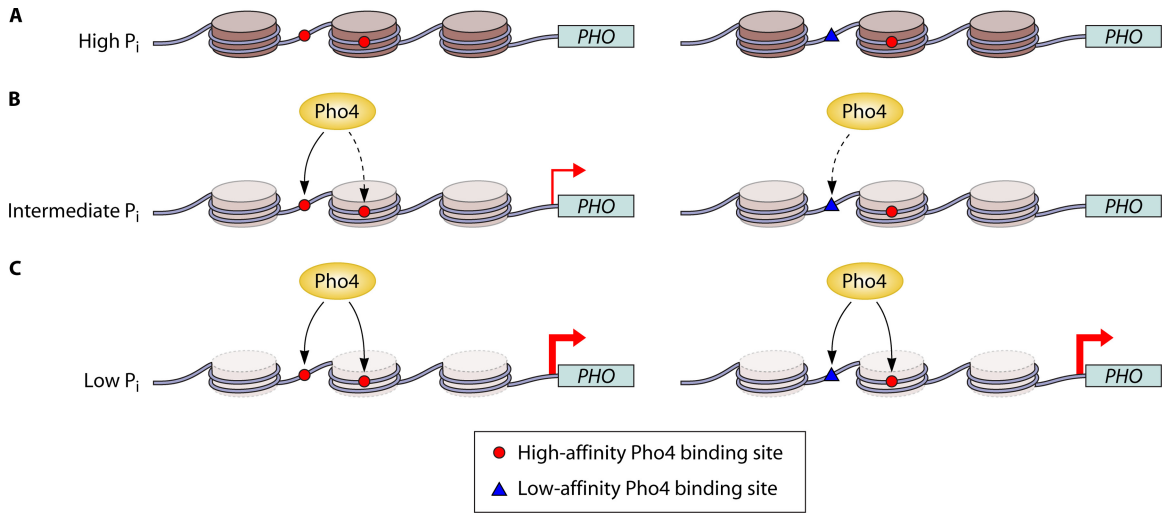

FIG. 4. Chromatin structure controls *PHO* gene expression in a dynamic manner. Depicted is a model describing a possible mechanism that decouples the induction threshold from the dynamic range. Nucleosome occupancy is indicated by the opacity of the ovals, and the amount of transcription is indicated by the thicknesses of the arrows. (A) In high levels of inorganic phosphate (P_i), nucleosomes are fully assembled on the *PHO* promoters, and the Pho4 transcription factor is absent, resulting in very low expression levels of the promoter. (B) At intermediate P_i concentrations, submaximal Pho4 transcription occurs. (Left) An exposed high-affinity site might be sufficient to allow for Pho4 binding. (Right) Promoters with an exposed low-affinity binding site do not obtain similar Pho4 binding because the Pho4 concentration is not sufficient to achieve a significant binding of this transcription factor. (C) Strong P_i starvation results in increased Pho4 concentrations, inducing the expression of promoters with exposed (low- or high-affinity) binding sites. Furthermore, the nucleosomes are evicted so that all Pho4-binding sites become exposed and accessible, leading to maximal expression. Expression is halted only after Pho4 levels drop significantly, which may give rise to hysteresis, especially in the promoter with an exposed low-affinity binding site and a covered high-affinity binding site (right). (For more details, see reference 90.)

whether the rearrangement of the nucleosome structure plays an important role in the regulation of the majority of genes. Genome-wide studies showed that chromatin remodeling events explain a large portion of the variation in expression between different *S. cerevisiae* strains (23, 94). As chromatin remodeling results in altered nucleosome positions, one wonders whether there is an overall correlation between nucleosome rearrangements and expression changes.

To answer this question, several genome-wide studies have set out to examine nucleosome occupancy and/or positions under conditions in which a subset of yeast genes undergoes a significant change in expression. Lee et al. (93) found that alterations in transcription caused by heat shock or a change in the carbon source resulted in increased nucleosome occupancy at repressed promoters and decreased nucleosome occupancy at promoters that became active. Similarly, other studies reported a correlation between nucleosome occupancy and transcription (96, 132). Tirosh et al. (175) found a strong correlation between changes in nucleosome positioning and the gene expression of ploidy-regulated genes. Here, the transcription of haploid-specific genes in a diploid strain was prevented by chromatin remodeling (175). Hogan et al. (65) showed that nucleosome occupancy at the promoters of some cell-cycle-regulated genes was reduced specifically at the cell cycle phase in which that gene exhibited peak expression. However, their study did not reveal large, global changes in nucleosome occupancy in response to cell cycle changes (65). Similarly, other studies failed to find strong correlations between changes in promoter nucleosome positions or occupancy and changes in expression, at least on a global scale. Shivaswamy et al. (159) examined nucleosome positioning before and after heat shock. Although those authors observed local nucleosome remodeling events at promoters, they found no general relationship between transcriptional changes and altered nucleosome positions (159). Zawadzki et al. (208) examined the global chromatin structure before and after glucose-induced transcriptional reprogramming, by which more than half of all yeast genes significantly change expression. Here, most induced and repressed genes did not exhibit an observable change in the promoter nucleosome arrangement (208). Another study also used alternative carbon sources and concluded that nucleosome occupancy remains largely unchanged (79). Taken together, these results indicate that the influence of nucleosomes on gene regulation is often not dynamic, at least not on the same time scale as that of regulation by TFs.

Nucleosomes Influence Adaptive Evolution of Expression Levels

Recent studies have used genome-wide transcription data to investigate which genes evolve more quickly in expression levels than others. One of the major conclusions is that genes containing a TATA box in their promoter evolve and diverge more quickly (172, 177). Interestingly, TATA boxes (which are present at 20 to 25% of all yeast genes) tend to cooccur with promoters lacking a clear NFR (4, 15, 68, 71, 173). Indeed, although most yeast genes (>95%) have a 5′ NFR, a minority of yeast promoters does not contain strong antinucleosomal sequences, and these promoters lack the characteristic 5′ NFR (20, 47, 171). A classic example of such a "covered" promoter

is the *PHO5* promoter discussed above. The cooccurrence of TATA boxes with covered promoters is not yet understood. However, it has been shown that TATA boxes themselves do not cause increased promoter nucleosome occupancy (43, 63, 158).

It is still unclear whether the increased nucleosome density plays a role in the evolutionary plasticity of TATA genes. TATA-containing genes are enriched for a variety of features compared to non-TATA-containing genes, which might be a result of the increased nucleosome occupancy observed at these promoters. One can speculate that various factors associated with the nucleosome occupancy in TATA promoters may affect how swiftly the transcriptional activity can be adapted during evolution. For example, TATA genes contain more TF-binding sites than do TATA-less genes (91, 177). In addition, non-TATA-containing genes are more likely to recruit TATA-binding protein (TBP) to their promoter using TFIID, whereas TATA-containing promoters rely on a different TBP-containing complex termed SAGA (regardless of the presence of a TATA box, the transcription initiation factor TBP is present at all Pol II promoters) (15, 68, 95).

Studies have also shown that TATA genes are more likely to be sensitive to mutations of chromatin regulators, including chromatin remodeling complexes (23, 171). TATA boxes in covered promoters will often reside inside the edge of the +1 nucleosome. This occlusion of the TATA box by a nucleosome is thought to explain the need for the chromatin remodeling activity. In the case of the *PHO5* promoter, moving the TATA box only a few bases inside or outside the nucleosome edge greatly changes the promoter's dependency on chromatin remodelers (108). In contrast, for the relatively few open promoters that contain a TATA box, the TATA sequence is located close to the TSS and clearly within the NFR, making it easily accessible (e.g., the *CYC1* promoter) (107).

The frequent presence of nucleosomes over TF-binding sites might explain why TATA-containing, covered promoters exhibit high levels of transcriptional noise (i.e., cell-to-cell variability of gene expression within one population of cells) (120, 141). In TATA-containing promoters, TFs and nucleosomes compete for the same stretches of DNA, which is expected to contribute to the cell-to-cell variability in the expression of the downstream genes (18, 171). Binding-site exposure might allow TF binding followed by the induction of expression (24, 47, 171). Subsequently, the random release of TFs from their binding sites would permit nucleosome reassembly, thereby introducing a delay between transcription bursts and the generation of transcriptional noise. Indeed, studies of cell-to-cell variability in the *PHO5* promoter implicate nucleosome dynamics as a major contributor to expression variability (18, 140). Such expression noise may provide variation on which natural selection can act, thereby possibly promoting adaptive evolution (139). On the other hand, the accessibility of TF-binding sites in (TATA-less) open promoters would allow for the steady reinitiation of transcription, resulting in low levels of transcriptional noise. Housekeeping genes, which would benefit from continuous expression, tend to be TATA-less. In contrast, TATA-containing genes are generally highly regulated stress-responsive genes (15).

A mechanism that connects nucleosome occupancy and the swift evolution of transcription is the occurrence of tandem-repeat tracts in about 20% of all promoters (see above). These tandem repeats are often extremely AT rich and seem to function as antinucleosomal barriers that help to establish a 5′ NFR in these promoters. Interestingly, tandem repeats are inherently unstable, and natural variation in the number of repeated elements causes variation in the 5′ NFR. This in turn generates variation in expression, upon which natural selection can act. In other words, unstable tandem repeats seem to constitute hypervariable and evolvable antinucleosomal barriers that promote the rapid adaptation of gene expression (188).

NUCLEOSOME POSITIONING IN OTHER EUKARYOTES

S. cerevisiae has been the model organism of choice in the majority of genome-wide studies examining nucleosome positions and the signals directing chromatin architecture. However, several research efforts have been devoted to unraveling the genome-wide nucleosome organization of other organisms, including other *Hemiascomycota* yeast species (175, 181), fission yeast (85, 92), *Plasmodium falciparum* (194), worms (76, 184), flies (110), medaka (Japanese killifish) (148), and humans (14, 31, 126, 150).

Universal Organization of Nucleosomes

For the majority of the eukaryotes studied to date, the same pattern of nucleosome organization on genes has been observed, including a general depletion of nucleosomes over promoter regions (except in humans [see below]), allowing for the binding of the transcription machinery. Consistent with the statistical positioning of nucleosomes, arrays of regularly spaced nucleosomes occur in the coding regions. In addition, for flies and all *Hemiascomycota* yeast species, a 3′ NFR was also detected (110, 175, 181). Thus, the general principles of chromatin organization have been conserved throughout the species studied to date.

In addition to these similarities, some interesting differences in nucleosome organizations between species should be noted. The average linker lengths (~18 bp in *S. cerevisiae* [96, 109, 159]) measure ~7 bp in *Schizosaccharomyces pombe* (92), ~28 bp in *Drosophila melanogaster* (110) and *Caenorhabditis elegans* (184), and ~38 bp in humans (14, 150). In medaka, nucleosomes most commonly are separated from each other by an 18-bp linker, while a distinctive ~200-bp spacing (~50-bp linker) is seen downstream of TSSs. Tsankov et al. (181) reported the variation in nucleosome spacing in coding regions for 12 *Hemiascomycota* species, with median nucleosome-to-nucleosome distances ranging between 160 bp and 177 bp. Their data indicated that linker length varies significantly even between closely related species.

The position of the +1 nucleosome relative to the TSS varies between organisms, likely reflecting differences in transcription regulation. In *S. cerevisiae*, transcription starts ~10 bp into the +1 nucleosome (4, 93, 96, 109, 207), whereas in metazoans, the upstream border of this nucleosome is located ~60 bp downstream of the TSS (14, 110, 184). In human T cells, the +1 nucleosome appears to be located ~40 bp downstream of the TSS in actively transcribed genes, but this distance shortens to

~10 bp in inactive genes. Because Pol II is also localized primarily at around ~10 bp, this shift in the position of the +1 nucleosome might be caused by Pol II binding in active genes (14).

The DNA-encoded signals directing nucleosome organization have been conserved across species. Nucleosome formation is facilitated by a 10-bp periodicity of A/T dinucleotides offset with a similar 10-bp pattern of G/C (76, 110, 184). However, the major *cis*-encoded nucleosome-positioning signal originates from AT-rich sequences and, specifically, poly(dA:dT) tracts that act by strongly preventing nucleosome formation (170, 181). Nucleosome exclusion by poly(dA:dT) sequences appears to be universal, although one exception has been reported so far: in *S. pombe*, poly(dA:dT) sequences occur less frequently in NFRs than elsewhere in the genome (92).

In contrast to *S. cerevisiae* promoters and regulatory sequences, which tend to be devoid of nucleosomes, human promoters, enhancers, and TF-binding sites often show high-level nucleosome occupancy *in vivo* (170). These regions tend to be devoid of poly(dA:dT) tracts in humans but instead have high GC contents, indicating that the nucleosome preference of these elements is intrinsically encoded in the DNA sequence. As is the case for *S. cerevisiae*, it was suggested that nucleosome-occupied promoters offer additional levels of specificity in gene regulation, which would allow for the cell-type-specific control of gene expression (170).

Nucleosomes and the Evolution of Gene Regulation across Yeast Species

Recently, the relationship between nucleosome positions and (the evolution of) gene expression in closely related yeast species has been explored (175, 181). Tirosh et al. (175) examined *S. cerevisiae* and its close relative *Saccharomyces paradoxus* for changes in nucleosome positions and concluded that ~70% of the differences observed between these two strains are due to changes in DNA sequences (i.e., in *cis*). Those researchers also examined gene expressions in both strains and found that transcription regulation is robust in response to many variations in nucleosome positioning, with the exception of changes in critical regulatory elements (174, 175).

Field et al. (46) examined evolutionary changes in gene expression and DNA-encoded promoter nucleosome occupancy in two closely related species, *S. cerevisiae* and *Candida albicans*. In high glucose, *C. albicans* grows by respiration, whereas *S. cerevisiae* grows primarily by fermentation. Upon an examination of the respiration genes, those authors found that in *C. albicans* (where these genes are active), the promoter sequences of these genes encode a relatively open (nucleosome-depleted) chromatin organization. In contrast, in *S. cerevisiae*, the promoter exhibits a closed (nucleosome-occupied) structure. Here, the respiration genes are inactive under typical growth conditions. Thus, those authors demonstrated a correlation between the differential expression of respiration genes and the nucleosome occupancy of these genes (46, 69).

In their study of 12 *Hemiascomycota* yeast species, Tsankov et al. (181) found evidence for both a conservation and a divergence of chromatin organization, which can be linked to the evolution of gene expression. Expression levels, nucleosome-excluding sequences, and binding sites for general regulatory factors that recruit chromatin remodelers were identified as the key determinants of NFRs. The interplay between these factors controls nucleosome positions in promoters, and the balance between these three contributors changes in evolution (181).

CONCLUSIONS

The recent availability of genome-wide nucleosome maps has revolutionized our understanding of the factors that affect where nucleosomes are formed and how this in turn influences and regulates various cellular processes. These novel findings paint a complex picture of the intricate interplay between the DNA sequence, various regulatory processes, and the three-dimensional chromatin structure and add another layer to the complexity of the genome architecture.

In *S. cerevisiae*, three factors emerge as major regulators of nucleosome positioning (Fig. 5). First, the local DNA sequence plays a significant role (Fig. 5A). In particular, AT-rich stretches of DNA seem to act as antinucleosomal sequences that are often flanked by highly positioned nucleosomes. Second, in turn, such highly positioned nucleosomes affect the positioning of neighboring nucleosomes, much as a fixed bead influences the positioning of neighboring beads on the same string. These two *cis* factors are incorporated into the barrier model for nucleosome positioning. Third, *trans* factors such as chromatin-modifying enzymes can move or eject nucleosomes or can change the stability of specific nucleosomes through chemical modifications of the histone tails (Fig. 5B).

Apart from improving our understanding of the factors that affect nucleosome positioning, the recent genome-scale studies of *S. cerevisiae* have also shed new light on the biological function of nucleosomes. Clearly, nucleosomes are much more than just a way to help pack DNA into the confined space of the nucleus. Over evolutionary time scales, genomes have evolved intricate signals to direct nucleosome positioning, which is in turn exploited to ensure the proper regulation and fine-tuning of various critical cellular processes.

It is important that nucleosome positioning is a highly dynamic process. Whereas many nucleosomes are highly positioned and do not seem to change between different (genetically identical) populations, or under different conditions, some nucleosomes are less stable and seem to be positioned differently in different cells within a population. In addition, *trans* factors can actively modify or move nucleosomes. This dynamic nature of nucleosome positioning helps to regulate processes like gene expression and can also introduce cell-to-cell variability upon which natural selection can act, thereby facilitating adaptive evolution.

In the model eukaryote *S. cerevisiae*, the interplay between the DNA structure and regulatory and evolutionary processes is now slowly being uncovered. However, although many of the general features and principles are conserved in higher eukaryotes, it is likely that other organisms have evolved different variations and mechanisms. Much more work will be needed to uncover the complete depth and

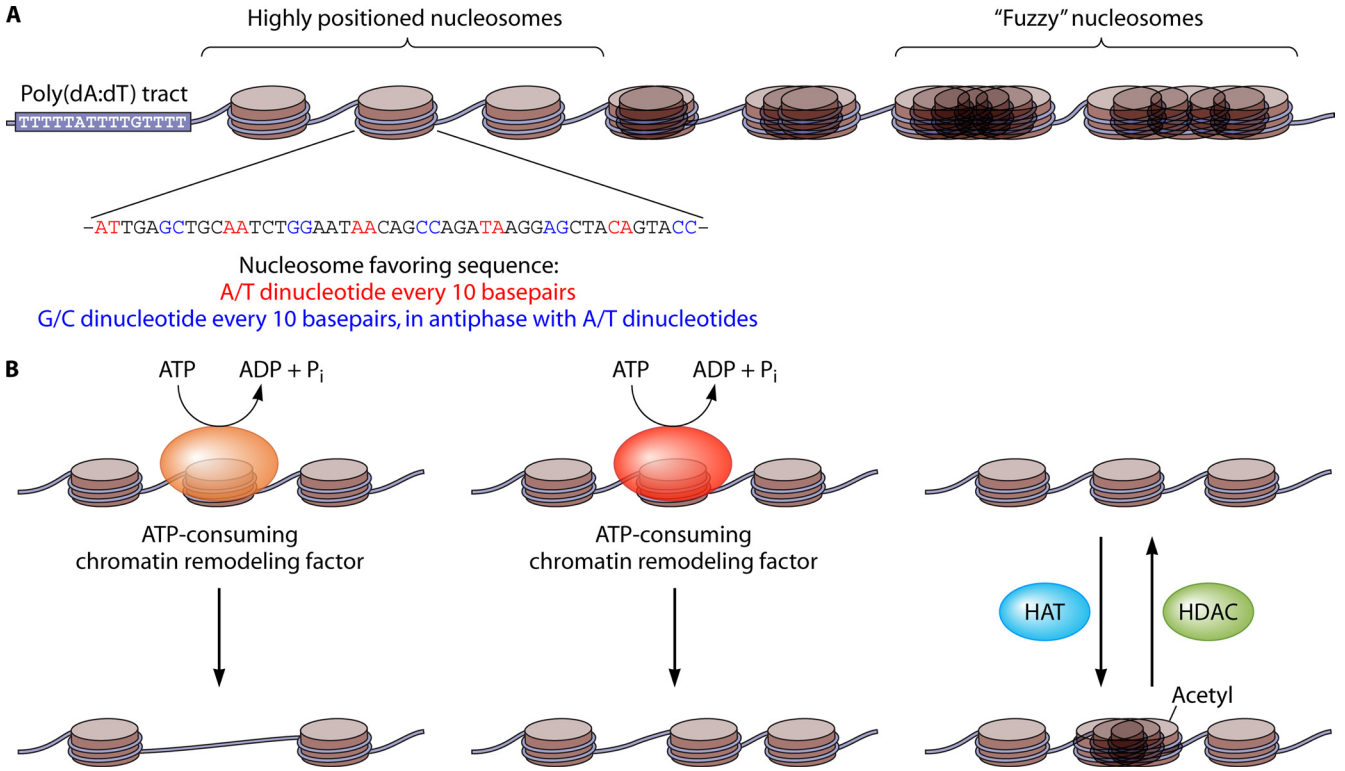

FIG. 5. *cis* and *trans* factors affecting nucleosome positioning in *S. cerevisiae*. (A) The most important *cis* factor affecting local nucleosome positioning is the AT content of the local DNA. Poly(dA:dT) tracts act as antinucleosomal "barriers." These barriers do not form a nucleosome, but highly positioned nucleosomes are often formed immediately adjacent to the barrier element. Second, the formation of nucleosomes is further enhanced by DNA sequences containing regularly spaced A/T dinucleotides (approximately one dinucleotide every 10 bp) and G/C dinucleotides (in between the A/T dinucleotides). Third, nucleosomes are also positioned by the steric hindrance of neighboring nucleosomes, much like beads on a string. This positioning signal is not perfect and deteriorates with increasing distance from strongly positioned nucleosomes, explaining why nucleosome positioning becomes increasingly "fuzzy" with increasing distance to positioning signals. (B) *trans* factors like ATP-consuming chromatin remodeling factors can remove (left) or slide (middle) nucleosomes. In addition, histone-modifying enzymes can add or remove covalent modifications to certain histone residues. For example, histone acetyltransferases (HATs) can add an acetyl residue, which can be removed by histone deacetylases (HDACs). Acetyl groups add an electronegative charge to the histones, which repulses the negatively charged DNA polymer, resulting in a modified, "looser" DNA-histone interaction and an increased accessibility of the DNA.

complexity of the relationship between genomes, nucleosomes, and cellular physiology.

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